Effect of Hypokalemia and Hypomagnesemia Produced by Hemodialysis on Vascular Resistance in Canine Skeletal Muscle

ROLE OF POTASSIUM IN ACTIVE HYPEREMIA

By D. K. Anderson, S. A. Roth, R. A. Brace, D. Radawski, F. J. Haddy, and J. B. Scott

ABSTRACT

Hemodialysis was used to study the effect of an acute local decrease in plasma $[K^+]$ or $[Mg^{2+}]$ or in both on vascular resistance in skeletal muscle. A dialyzer was placed in the arterial supply of the collateral-free gracilis muscle of the dog and blood flow was held constant while measuring perfusion pressure. Pressure increased linearly with decreased $[K^+]$ down to 0.2 mEq/liter. A 50% decrease in $[K^+]$ produced a 12% increase in resistance. Prolonged hypokalemia produced a sustained increase in perfusion pressure and a decreased responsiveness to close arterial injection of norepinephrine. Removal of up to 84% of the plasma $Mg^{2+}$ produced no effect, either alone or in conjunction with hypokalemia. When the potassium level of the perfusing blood was changed from normal to hypokalemic during the dilation brought on by simulated exercise, the resistance did not change. In addition, the magnitude of the resistance changes seen during exercise were much greater than could be induced by local changes in plasma $[K^+]$ alone. It is concluded that hypokalemia produces active constriction of vascular smooth muscle. However, this study fails to lend support to the idea that potassium alone is responsible for exercise dilation.

KEY WORDS: exercise hyperemia gracilis muscle norepinephrine potassium depletion osmolality

The effect of alterations in plasma ionic concentrations, both above and below normal concentrations, on resistance to blood flow through various systemic organs has been the subject of a great deal of research. Increasing the blood concentration of most ions is conveniently accomplished by intra-arterial infusion of the ion in an isosmolar solution. Decreasing the blood concentration of a single ion without producing any other change is difficult. In a previous study (1) we employed a dilution technique to reduce ion concentration, and data from that study suggest that reduction of plasma $[K^+]$ results in active constriction in canine forelimb and kidney, whereas low plasma $[Mg^{2+}]$ is without effect. However, the dilution technique used in that study to produce the concentration changes has some disadvantages. Dilution produces secondary changes in other variables—for example, hematocrit, blood viscosity, protein binding of cations, nonelectrolyte concentrations—making it necessary to infuse a control solution that produces all the changes except that under study. The results must then be interpreted in light of the effect of the control solution. Also, reduction of ion concentrations to very low levels requires great dilution.

Hemodialysis, the technique used in the present study, allows transfer of selected ions...
to and from blood with little or no change in hematocrit, viscosity, and plasma protein concentration. The dialysate fluid, hence the concentration of selected ions in the perfusing blood, can be changed by switching to a different dialysate supply with no disturbance of blood flow because the blood and dialysate streams are physically separate. With this technique, the constrictor effect of hypokalemia and the absence of response to hypomagnesemia were confirmed for the dog gracilis muscle in situ but freed from collateral circulation. The increase in vascular resistance above control was determined as a function of the level of induced hypokalemia. In addition, the role of K⁺ in exercise hyperemia was studied by combined motor nerve stimulation and hemodialysis. Finally, the effect of potassium depletion on the response of the gracilis muscle to norepinephrine was examined.

Methods

Dogs weighing 20–40 kg were anesthetized by injection of sodium pentobarbital (33 mg/kg, iv) and ventilated with a mechanical positive pressure respirator via an intratracheal tube. The right hindlimb gracilis muscle was surgically exposed and isolated from the body between its origin and insertion except for the main gracilis artery, vein, and nerve. The origin and insertion were then ligated (for detailed procedure, see ref. 2). This was followed by an injection of sodium heparin (5 mg/kg, iv). A cannula was placed in a side branch of the gracilis vein to allow sampling of venous blood. The left femoral artery was ligated and a constant displacement blood pump was interposed between the proximal segment of the femoral artery and the hemodialyzer. The dialyzer was flushed with saline and then filled with arterial blood. Blood leaving the dialyzer entered the gracilis artery and blood flow rate was adjusted so that the perfusion pressure was approximately equal to systemic pressure. Flow rate ranged from 5 to 25 ml/min in different experiments, depending on muscle size and initial resistance, but was maintained constant in any given experiment. Inlet and outlet dialyzer pressures and perfusion and systemic pressures were monitored continuously on a direct-writing oscillograph. Four pressure transducers were used and all tubes and needles were flushed periodically with heparinized saline.

Two parallel-plate dialyzers similar in design to that developed by Babb and Grimsrud (3–5) as an artificial kidney were used. In this design, the Cuprophane PT 150 membrane is supported by foam nickel metal.¹ The porous metal allows the dialyzer fluid to flow through its structure while maintaining rigid support for the membrane. The dialyzer design is illustrated in Figure 1.

Blood concentration changes were achieved by using different dialysate solutions. The control dialysate was a modified Ringer's solution (concentration in mEq/liter): Na⁺, 146; Mg²⁺, 2; K⁺, 4; Ca²⁺, 5; Cl⁻, 131; HCO₃⁻, 21; osmolality, 300). For the hypokalemia experiments, the K⁺ was replaced with Na⁺, and for the hypomagnesemia experiments, Mg²⁺ was replaced with Na⁺. For the combination of hypokalemia and hypomagnesemia, both K⁺ and Mg²⁺ in the dialysate were replaced with Na⁺. The dialysate solution volume (6 liters) was sufficiently large so that at no time did either the K⁺ or Mg²⁺ in the dialysate (dialyzed from the blood) reach 10% of the blood concentration. All solutions were maintained at 37°C.

The gracilis muscle was perfused with blood dialyzed against the control solution until pressure was steady; samples were then drawn from the arterial blood entering and leaving the dialyzer, and venous blood leaving the gracilis muscle. By means of a valving arrangement, the control solution was changed to the dialysate solution lacking the ion(s) of interest. After a new steady-state perfusion pressure had been reached, additional blood samples were taken. The samples were analyzed for plasma potassium and for magnesium concentrations. Osmolality, pH, and hematocrit were checked periodically and found to be unchanged by the dialyzer.

The experimental program consisted of dialyzing the blood alternately against the control dialysate and the dialysate with no K⁺ or no Mg²⁺ or neither (5–10 minutes) and calculating the change in vascular resistance from the steady-state perfusion pressure and blood flow. In addition, the experimental procedure included challenging the muscle for 1 hour with blood dialyzed against the zero potassium dialysate to determine if the acute response to hypokalemia was altered with time. After 1 hour of hypokalemic perfusion, the dialysate was switched to normal Ringer’s solution. In addition, the effect of potassium depletion on the vascular response to norepinephrine was examined by injecting 0.1 ml of a solution of norepinephrine (concentration 1µg/ml) into the blood perfusing the muscle when dialyzed against normal Ringer’s solution and comparing this response to those produced by norepinephrine injections at 5, 30, and 45

¹Available commercially from General Electric Company, Detroit, Michigan.
minutes into the 1-hour period of potassium depletion.

In a second series of experiments, the gracilis nerve was faradically stimulated for 3 minutes to induce active dilation. At 2.5 to 3.0 minutes of stimulation, the dialysate was switched from the control to the K⁺-free Ringer’s solution and the muscle was perfused with hypokalemic blood for 1 hour. The muscle was then stimulated again for 3 minutes, hypokalemic perfusion continuing.

Arterial and venous samples were taken before and during each stimulation. These were analyzed for [K⁺] and osmolality.

Results

The amount of potassium dialyzed from the blood depended mainly on the blood flow rate and the transfer area available in the dialyzer. Blood flow rates to the gracilis varied from

FIGURE 2

Systemic pressure and perfusion pressure at constant flow when blood was changed from normo- to hypokalemic.

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about 5 to 25 ml/min and the two dialyzers used had transfer areas of approximately 200 and 1000 cm². The combination of the lower flow rate with the larger dialyzer made it possible to remove in excess of 95% of the normal potassium from the perfusing blood.

Figure 2 is a tracing from a typical hypokalemia experiment. The arrow indicates the point at which the dialysate solution was switched from the control Ringer's solution to one containing zero potassium. After a time lag of about 1 minute, muscle perfusion pressure began to rise and leveled off in another 2 minutes. The absence of an immediate rise in perfusion pressure after switching dialysate sources is attributed mostly to the fact that the gracilis muscle was still perfused with normokalemic blood from the connecting tubing and outlet of the hemodialyzer.

Figure 3 summarizes the results of the short-term hypokalemic perfusion on the gracilis muscle vasculature. For the range of plasma potassium concentrations considered, approximately 0.2 to 4 mEq/liter, the data were fit to the straight line

\[ \frac{P_e - P_c}{P_c} = -0.25 \left( \frac{K_e - K_c}{K_e} \right) \]

with a coefficient of correlation of 0.90. P and K are the perfusion pressure and plasma \([K^+]\) entering the muscle, respectively. Subscripts e and c are the experimental and control values of the perfusion pressure and plasma \([K^+]\) entering the muscle. Each point represents an increase in perfusion pressure—hence vascular resistance—when switching from control to hypokalemic blood or a decrease in these parameters when switching from hypokalemic blood to control. No significant difference was obtained when the on and off responses were plotted separately. However, when the change in resistance was compared to the plasma \([K^+]\) leaving the muscle, there was less correlation (correlation coefficient = 0.20).

One hour of hypokalemic perfusion produced an increase in perfusion pressure above the short-term (5-10 minutes) response to hypokalemia in nine of ten experiments (Table 1). On returning to control Ringer's solution, pressure fell but remained well above control (37%). In 16 animals perfused for 1 hour
TABLE 1
Average Effects of Prolonged Hypokalemia on Perfusion Pressure at Constant Flow (n = 10)

<table>
<thead>
<tr>
<th>Time</th>
<th>Dialysate</th>
<th>Perfusion pressure</th>
<th>Percent above control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Control</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>5-10 min</td>
<td>K⁺-free</td>
<td>132</td>
<td>16</td>
</tr>
<tr>
<td>60 min</td>
<td>K⁺-free</td>
<td>167</td>
<td>46</td>
</tr>
<tr>
<td>65-70 min*</td>
<td>Control</td>
<td>156</td>
<td>37</td>
</tr>
</tbody>
</table>

*On normal Ringer's solution 5-10 min.

Hypokalemic blood, the response to norepinephrine was slightly depressed from that during perfusion with normal blood. The response was markedly depressed at 30 and 45 minutes of hypokalemic perfusion. Table 2 presents the averages of nine such experiments. The peak increase in perfusion pressure (ΔP) and the area under the pressure curve produced by norepinephrine were significantly decreased at each time interval. Although the decrease in response may be in part related to the increased resistance produced by hypokalemia, this cannot be the only factor. In two experiments, resistance was not above the control level at the end of 45 minutes of hypokalemic perfusion, yet the response to norepinephrine was reduced. Table 2 also shows that switching the dialysate to normal Ringer's solution did not reestablish the response to norepinephrine.

Figure 4 is a typical tracing showing the response of gracilis perfusion pressure to norepinephrine. Injection of 0.1 μg of norepinephrine during perfusion with normal blood produced an immediate increase in perfusion pressure. After 5 minutes of perfusion with hypokalemic blood, the response to norepinephrine was slightly depressed from that during perfusion with normal blood.
TABLE 2
Response to Norepinephrine Injections during Normal and Hypokalemic Perfusion (n = 9)

<table>
<thead>
<tr>
<th>Time</th>
<th>Dialysate</th>
<th>ΔP (mm Hg)</th>
<th>Area* (m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Control</td>
<td>29</td>
<td>253</td>
</tr>
<tr>
<td>5 min</td>
<td>K+-free</td>
<td>19</td>
<td>146</td>
</tr>
<tr>
<td>30 min</td>
<td>K+-free</td>
<td>19</td>
<td>128</td>
</tr>
<tr>
<td>45 min</td>
<td>K+-free</td>
<td>13</td>
<td>82</td>
</tr>
<tr>
<td>70 min†</td>
<td>Control</td>
<td>7</td>
<td>11</td>
</tr>
</tbody>
</table>

ΔP = maximum change of pressure after injection.
For all changes P < 0.05 when compared to response at time 0.
*Area under perfusion pressure response curve.
†On normal Ringer’s for 10 min (n = 6).

gracilis responded with a decrease in resistance initially, but failed to respond on further exposures to hypomagnesemia.

Three of the above experiments included simultaneously perfusing the gracilis muscle with hypokalemic and hypomagnesemic blood. On the average, removal of 65% of the Mg²⁺ and 86% of the K⁺ from the blood produced a 21% change in perfusion pressure. From Figure 3 it can be seen that this increase in perfusion pressure corresponds to an 86% decrease in plasma [K⁺]. Thus the [K⁺] decrease alone can account for the increase in pressure.

Figure 5 is a tracing of the pressure response when the gracilis nerve was stimulated (6 v, 1.6 msec, 6 cps) for 3 minutes during perfusion with normal blood. Just before stopping the stimulation, the dialysate was changed to K+-free Ringer’s solution. Thus hypokalemic blood entered the muscle approximately 1 minute after stimulation was terminated. Stimulation reduced perfusion pressure at constant flow and changing to hypokalemic blood at this time failed to measurably affect perfusion pressure.

The [K⁺] was determined in blood entering and leaving the muscle before, during, and after stimulation. The results from ten such experiments are shown in Table 3. Perfusion pressure initially dropped from 107 mm Hg to 74 mm Hg and returned gradually to the control level. The [K⁺] of the blood entering the muscle was decreased from 3.89 to 0.94 mEq/liter shortly after stimulation was stopped. Associated with the drop in arterial [K⁺] was a reduction in venous [K⁺] from 5.57 to 1.52 mEq/liter. These changes in [K⁺] were not associated with changes in vascular resistance.

Dialysis was continued for 1 hour or longer with the result that approximately 10% of the calculated cellular potassium was removed from the muscle tissue as estimated from arterial and venous [K⁺], blood flow rate, and contralateral gracilis muscle weights. By this time, resistance had returned only to control level as opposed to the nonstimulation experiments, in which the resistance was above control (Table 1). Stimulation resulted in the same decrease in resistance as it did initially and the same increment of rise in venous [K⁺]. However, the absolute level of venous potassium was greatly different in the two situations (Table 3). Venous osmolality increased to the same extent during both stimulation periods.

Discussion

The use of hemodialysis, as reported here, provides a very selective method of altering blood composition for the study of the local vascular effect of ions. The concentration of any one or more of the small ions can be reduced or increased rapidly in the blood perfusing a vascular bed with little or no effect on other variables such as osmolality, protein concentration, hematocrit, and flow rate. It is possible that other substances are lost during
dialysis; however, they would be lost to an equal extent during control perfusion. Furthermore, because of the low flow rates used, it is unlikely that the concentration of any substance was altered in the systemic blood.

**Potassium.**—These studies confirm that locally decreasing the [K⁺] of the blood perfusing a vascular bed increases resistance to flow (1). They do not, however, indicate whether the resistance change is a result of increased viscosity or decreased vessel caliber. In this regard, a parallel investigation by Masin (6) showed that increased [Mg²⁺] had no effect on blood viscosity but increased [K⁺] resulted in a slightly higher viscosity. This latter finding suggests that the resistance changes seen in the present study can be attributed to increases or decreases in the caliber of the vessels.

A mechanism by which vessel caliber might be involved is not immediately obvious, since one might expect decreased [K⁺], to result in hyperpolarization and dilation. One possible mechanism is that decreasing [K⁺] suppressed the Na-K-sensitive membrane ATPase, causing a decrease in the activity of an electrogenic Na-K pump. This results in a net increase of positive charge inside the cell (depolarization) and contraction. In fact it has been demonstrated recently that a decrease of potassium within the range of 0 to 8 mEq/liter in the fluid bathing certain tissues does result in depolarization rather than hyperpolarization (7). Preliminary studies in this laboratory lend further support to this hypothesis (8). Ouabain, a Na-K ATPase inhibitor, was injected intra-arterially into the gracilis muscle, and hemodialysis was then used to measure the potassium response. Ouabain abolished hypokalemic constriction and attenuated hyperkalemic dilation. It would thus seem that the constrictor and dilator actions of potassium may be related to its effect on ATPase.

The decreased response to norepinephrine seen during hypokalemic perfusion is in accord with the results reported for the rabbit ear perfused with modified Locke’s solution (9) and for isolated aortic strips (10) bathed in a medium with low [K⁺]. In the rat, a potassium-deficient diet lowers blood pressure (11), decreases myocardial contractile force (12), and increases the norepinephrine content in cardiac and splenic nerve terminals (13). On the other hand, an acute increase in [K⁺] in the perfusing blood also has been shown to decrease the response to norepinephrine (14, 15). The mechanism of these effects is unclear.

**Magnesium.**—The absence of a resistance change when [Mg²⁺] was lowered agrees with a previous study (1) using the dilution technique. One might have expected an effect since magnesium is a well-known dilator (16). Troponin, located in the thin filament, inhibits actomyosin contraction when [Mg²⁺] exceeds a threshold level and the sarcoplasmic reticulum ATPase and Na-K-sensitive membrane ATPase require magnesium (17-20). It has previously been reported that exposure of rabbit aortic strips to Mg²⁺-free Krebs-Ringer solution for 60 minutes increases the tension from 10% to 50% in 25% to 35% of the strips (17). In the present work, the smooth muscle cells were never exposed to Mg²⁺-free blood and exposure times were much shorter.
Perhaps we did not achieve enough depletion to affect the requirements of the system critically. Hence, the lack of response is not necessarily inconsistent.

**Exercise Hyperemia.**—When the perfusing blood was changed from normokalemic to hypokalemic during the dilation brought on by simulated exercise, the resistance did not change, even though the venous [K+] dropped from hyperkalemic (5.57 mEq/liter) to hypokalemic levels (1.52 mEq/liter). Furthermore, the magnitude of the resistance changes seen during exercise are much greater than can be induced by changing the [K+]; exercise caused a 32% decrease in perfusion pressure during hypokalemic perfusion while the venous [K+] increase can account for only a 10% change (based on the data of Figure 3). These findings do not support the idea that potassium alone can be responsible for exercise dilation. However, the study does not eliminate the possibility that potassium is involved in exercise hyperemia since potassium concentration does increase and potassium is a dilator. It has been suggested that various vasodilating influences interact or act in concert to produce the vascular dilation that accompanies skeletal muscle exercise (21, 22). In fact, Knockel and Schlein (23) reported that chronic dietary potassium depletion diminished active hyperemia when 25% of the cellular potassium was removed from the gracilis muscle. In addition, the venous potassium level failed to rise during stimulation after depletion. Both of these findings are in contrast to the present study, wherein the exercise dilation was not diminished and removal of 10% of the muscle potassium failed to prevent the rise in venous potassium during stimulation. In fact, the increase in venous [K+] was about the same as before depletion. These differences may be related to the method of potassium depletion (chronic vs. acute) or possibly to the extent of potassium depletion.

**References**


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